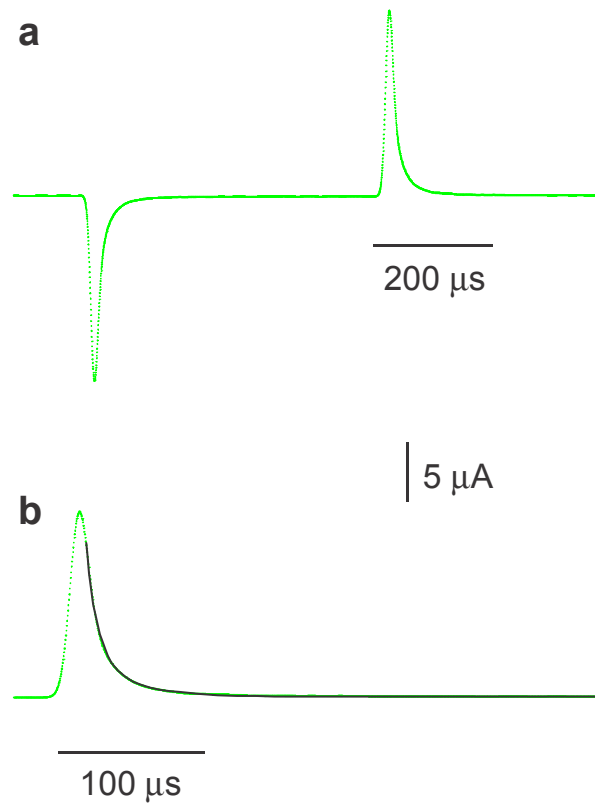
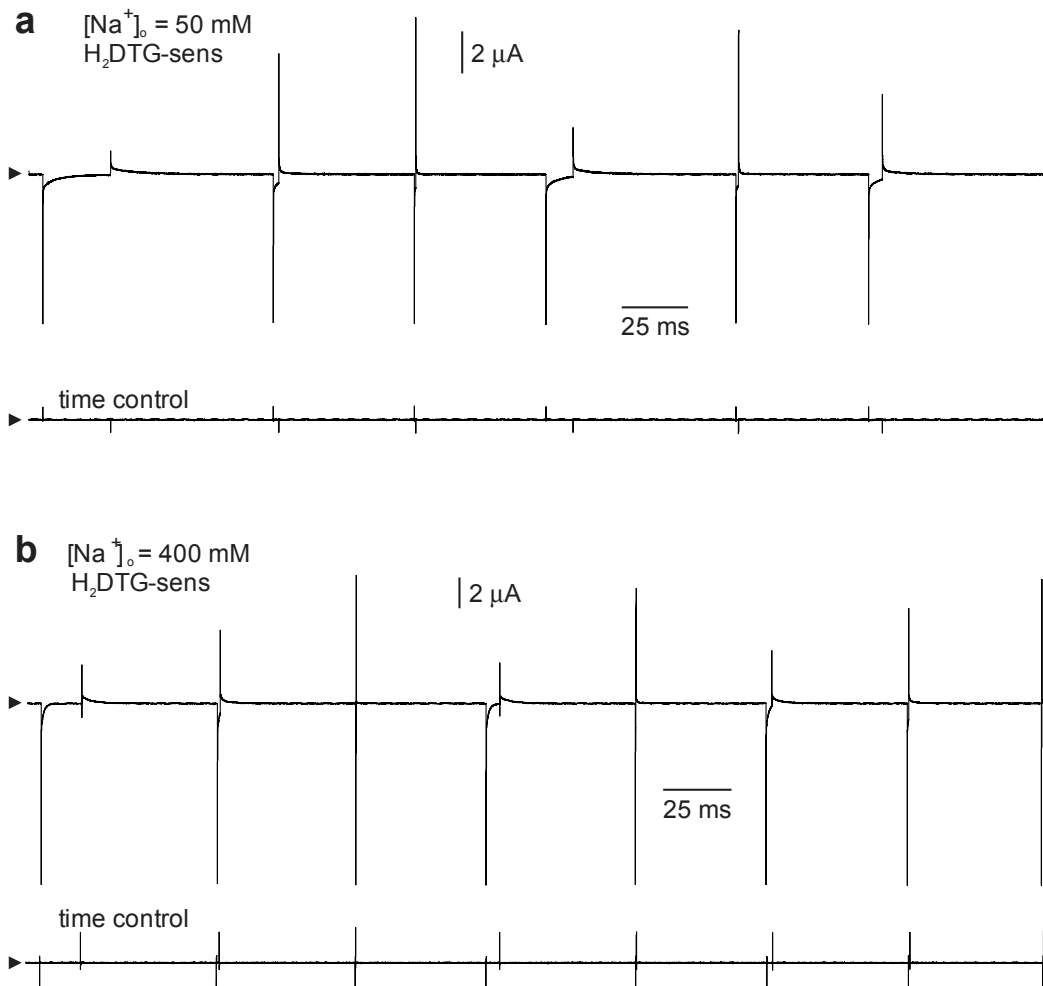


The dynamic relationships between the three events that release individual Na⁺ ions from the Na⁺/K⁺-ATPase.

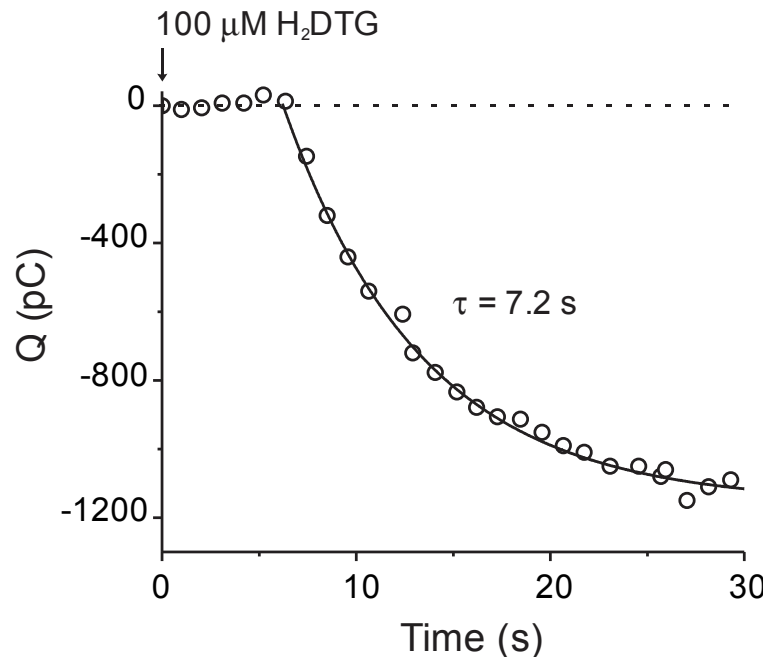
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Supplementary Figure S1. Speed of squid giant axon membrane voltage clamp. (a) Capacitance current transient from a representative axon in response to a 4 mV voltage step made after H₂DTG had been applied. (b) OFF membrane capacitance transient. The solid line represents a two-exponential fit to the current decay: the super-fast component, with a time constant of $7.46 \pm 0.05 \mu\text{s}$, was followed by a slower decay with $28.0 \pm 0.3 \mu\text{s}$ time constant. All axons studied had similarly fast membrane capacitance transients.



Supplementary Figure S2. H₂DTG-sensitive currents. H₂DTG-sensitive current traces, and corresponding time controls, from the experiments summarized in Figure 5 with $[\text{Na}^+]_o=50 \text{ mM}$ (**a**), and $[\text{Na}^+]_o=400 \text{ mM}$ (**b**). Time control error signals were consistently slightly larger at higher $[\text{Na}^+]_o$. These signals were assessed before application of H₂DTG and likely reflect small changes in membrane capacitance current over time, perhaps due to changes in series resistance within the Frankenhäuser-Hodgkin space²². In any case, they last only ~20-40 μs and represent <10% of the maximum Q_f ; all values of Q_f were corrected for these errors.



Supplementary Figure S3. H₂DTG action. Charge moved by the Na⁺/K⁺ pump was estimated by numerical integration of H₂DTG-sensitive currents in response to a 5 ms voltage step to -140 mV, from a holding potential of 0 mV, applied every ~1 s. H₂DTG (valve switched at arrow) takes ~6 s to reach the axon. The solid line represents a single exponential fit to the time course of H₂DTG-induced loss of charge. From 4 experiments, the mean τ for 100 μ M H₂DTG action was 6.7 ± 0.3 s. Two additional experiments using 50 μ M H₂DTG gave similar results ($\tau = 7.5 \pm 0.1$ s).